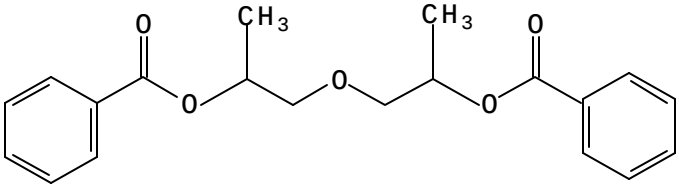
- Solvator for PVC
- Vinyl flooring
- Adhesives
- Plasticizers in elastomers
- Latex caulks and sealants
- Color concentrates for PVC
- Castable polyurethanes

Synonyms:

- CASRN: 27138-31-4
- Benzoflex® 9-88
- Benzoflex® 9-88 SG
- Propanol, oxybis, - dibenzoate
- Oxydipropyl dibenzoate
- Oxybispropanol dibenzoate

The structural formula is:



Dipropylene glycol dibenzoate is a clear colorless liquid with a mild ester odor.

Other typical properties include:

Relative Density:	1.12
Density, lb/gal at 25ºC	9.346
Henry's Law Constant	3.8 X 10^{-8} atm.m^3/mol
Flash Point (PMCC)	192ºC

Not explosive or pyrophoric

References:

Velsicol Chemical Corporation: Product Information Bulletin. Benzoflex 9-88. VCC97R01.

Benzoflex 9-88. Determination of Physico-Chemical Properties. Huntingdon Life Sciences. 1999

TEST PLAN

Study	Data Available	Data Acceptable	Testing Required
Melting Point	Yes	Yes	No
Boiling Point	Yes	Yes	No
Vapor Pressure	Yes	Yes	No
Partition Coefficient	Yes	Yes	No
Water Solubility	Yes	Yes	No
Photodegradation	Estimation	Yes	No
Stability in Water	Estimation	Yes	No
Transport (Fugacity)	Estimation	Yes	No
Biodegradation	Yes	Yes	No
Acute Toxicity to Fish	Yes	Yes	No
Acute Toxicity to Aquatic Invertebrates	Yes	Yes	No
Acute Toxicity to Aquatic Plants	Yes	Yes	No
Acute Toxicity	Yes	Yes	No
Repeated Dose Toxicity	Yes	Yes	No
Developmental Toxicity	Yes	Yes	No
Reproductive Toxicity	Yes	Yes	No
Genetic Toxicity Gene Mutation	Yes	Yes	No
Genetic Toxicity Chromosome Aberrations	Yes	Yes	No

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Dipropylene Glycol Dibenzoate
Robust Summary

BOILING POINT

Test Substance: Benzoflex 9-88 is a clear colorless liquid.

Purity Profile:

Dipropylene Glycol Dibenzoate:	89.4%
Dipropylene Glycol Monobenzoate:	4.98%
Propylene Glycol Dibenzoate:	2.29%
Propylene Glycol Monobenzoate:	0.28%
Propenyloxy Propyl Benzoate:	2.35%

Test Method: OECD 103, EEC A2

GLP: Yes
Year Performed: 1997

Results: Decomposes above 270°C without boiling at 762 mmHg

Data Quality: 1, Reliable without restrictions

References: Benzoflex 9-88. Determination of Physico-Chemical Properties. Huntingdon Life Sciences. 1999

VAPOR PRESSURE

Test Substance: See boiling point for purity.

Test Method: OECD 104, EEC A4

GLP: Yes
Year Performed: 1997

Results: 1.2×10^{-6} mm Hg @ 25°C
1.1×10^{-5} mm Hg @ 50°C
3.8×10^{-4} mm Hg @ 100°C

Data Quality: 1, Reliable without restrictions

References: Benzoflex 9-88 Determination of Physico-Chemical Properties. Huntingdon Life Sciences. 1999

Other Studies: Butz RG, Atallah YH, Yu CC and Calo CJ. Environmental Toxicology and Chemistry. 1, 337. 1982. Vapor pressure 2.29×10^{-7} mmHg at 25°C.

PARTITION COEFFICIENT

Test Substance: See boiling point for purity.

Test Method: OECD 117, EEC A8

GLP: Yes
Year Performed: 1997

Results: log P_{ow} = 3.9

Data Quality: 1, Reliable without restrictions

References: Benzoflex 9-88 Determination of Physico-Chemical Properties. Huntingdon Life Sciences. 1999

Other studies: Butz RG, Atallah YH, Yu CC and Calo CJ. Environmental Toxicology and Chemistry. 1, 337. 1982. K_{ow} – 455.

WATER SOLUBILITY

Test Substance: See boiling point for purity.

Test Method: OECD 105, EEC A6

GLP: Yes
Year Performed: 1997

Results: 8.69 mg/l @ 30°C, pH = 7.0 low solubility

Data Quality: 1, Reliable without restrictions

References: Benzoflex 9-88 Determination of Physico-Chemical Properties. Huntingdon Life Sciences. 1999.

Other Studies: Butz RG, Atallah YH, Yu CC and Calo CJ. Environmental Toxicology and Chemistry. 1, 337. 1982. Water solubility 15 mg/l at 25°C.

BIODEGRADATION

Test Substance: See boiling point for purity.

Test Method: OECD 301B

GLP: Yes
Test Type: aerobic
Year Performed: 1997
Laboratory: Huntingdon Life Sciences

Inoculum: Activated sludge from sewage treatment works
Concentration: 10 mgC/l
Duration: 29 days
Positive Control: Sodium benzoate
Control and Blank: Mineral salts and medium

Results: 6% of TCO_2 @ 2 days
62% of TCO_2 @ 12 days
85% of TCO_2 @ 28 days
Final mean level of degradation including residual CO_2 released from the medium after acidification.

Readily biodegradable.

Data Quality: 1, Reliable without restrictions

References: Benzoflex 9-88 Assessment of Ready Biodegradability - Modified Sturm Test. Huntingdon Life Sciences. 1998.

Other studies: Benzoflex 988: Assessment of biotic/abiotic degradability. Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD). Huntingdon Life Sciences. 1998. OECD 301D, Closed Bottle test. The BOD_5 of Benzoflex 9-88 was 0.65 gO_2/g (30% of its ThOD; 2.15 gO_2/g) based on results obtained at a nominal concentration of 2 mg/L.. The mean COD of Benzoflex 9-88 (2.33 gO_2/g) was 104% of its ThOD which confirmed that the material was completely oxidized in the COD test. The mean BOD_5 of Benzoflex 9-88 was 29% of its COD. For screening purposes, substances are generally considered readily biodegradable in this test if the ratio of BOD_5:COD or ThOD $\geq$ 50%. Benzoflex 9-88 cannot therefore be considered to be readily biodegradable under the conditions of this test. Because this type of BOD test employs both a weak microbial inoculum and a relatively short incubation time, it can be considered to be a particularly stringent test of biodegradability.

Benzoflex 9-88. Evaluation of Ultimate Anaerobic Biodegradability by Measurement of Biogas Production. Huntingdon Life Sciences. 1998. USEPA Method 796.3140, Anaerobic Biodegradability. Benzoflex 9-88 was degraded to 46% after 60 days of incubation and 75% after 120 days of incubation, based on a nominal level of carbon in the culture at the start of the test (12 mgC). At Day 120 of the test, Benzoflex 9-88 was degraded to 90% based on the theoretical carbon level (10 mgC) remaining in cultures following removal of samples for DIC analysis. The precise distribution of Benzoflex 9-88 in test mixtures was not determined in this test so the level of carbon remaining in test mixtures after samples were removed for DIC analysis cannot be accurately determined. However, since the octanol:water partition coefficient for Benzoflex 9-88 is relatively high (log P_{ow} 3.9), it is likely that the material will adsorb onto sewage solids. Although the level of biodegradation calculated using the nominal level of carbon at the start of the test (12 mgC) gives the worst case estimate, it is likely to be the most accurate. Substances are considered to be ultimately degraded under anaerobic conditions in this test if the level of degradation is equal to or greater than 60%. Benzoflex 9-88 can therefore be considered ultimately biodegradable under anaerobic conditions.

Peterson OM. Velsicol Chemical Corporation. 1974. The standard BOD test for biodegradability showed the oxygen consumed in 5 days was sufficient for the degradation of 26% of the compound, while in 20 days, the corresponding value was 84%.

Butz RG, Atallah YH, Yu CC and Calo CJ. Environmental Toxicology and Chemistry. 1, 337. 1982. ^{14}C-labeled Benzoflex 9-88 was virtually removed by 28 days and 78% of the carbon was converted to carbon dioxide. The remainder was incorporated into

biochemical components of the microorganisms, a normal occurrence for substances used as a food source by microorganisms.

ACUTE TOXICITY TO FISH

Test Substance:

Dipropylene Glycol Dibenzoate:	27138-31-4	98.95%
Unknowns 1-6:	Unknown	0.66%
Propylene Glycol Dibenzoate:	19224-26-1	0.28%
Dipropylene Glycol Monobenzoate:	32686-95-8	0.09%

Method

Guideline	OECD 203
Test Type	Acute Toxicity to Fish
GLP	Yes
Year	2000
Species/Strain	Fathead minnow (Pimephales promelas)
Analytical Monitoring	Every 24 hours
Exposure Period	96 hours
Test Details	Continuous flow
Statistical Methods	EC_{50} and 95% confidence limits were calculated by full probit regression using LOGIT Genstat. The program calculates the EC_{50} values using a logistic model (Berkeson, 1944) for which 95% confidence limits were estimated by the likelihood ratio method (Williams, 1986).

Berkson, J. (1944) Application of the logistic function bio-assay. *J. Amer. Statist. Assoc.* **39**, 357-365.

Williams, D.A. (1986) Interval Estimation of the Median Lethal Dose. *Biometrics* **42**, 641-645.

Test Condition Remarks

Fish Size and Age: Juvenile Pimephales promelas mean standard length 2.7 cm (mean total length >3.0 cm)

Test Conditions: As specified in OECD 203

Diluent Water Source and Chemistry: Laboratory tap water filtered, dechlorinated and softened by passage through an Elga® water purification system. Chlorine levels ranged form 0.01 to 0.08 mg/L throughout the 14 days of the acclimatization period and the exposure period and the hardness level, calculated from daily measurements during the same period was between 133 and 162 mg CaCO3/L.

Stock and Test Solutions: The test substance was dissolved in dimethyl formamide (DMF) to give a series of stock solutions at nominal concentrations of 2.5, 5.5,12, 27 and 58 mg/ml.. The stock solutions were dispensed automatically by the dosing apparatus into the flowing test water to give the desired series of test concentrations. The stock solutions were prepared twice during the exposure period and stored at approximately 4°C until required.

Vessels and Lighting: Five test concentrations plus one control and one solvent control were prepared (100µg/L auxiliary solvent per liter), each in duplicate. The vessels contained prepared test medium or diluent water plus 100µg/L auxiliary solvent per liter, or diluent water only, as appropriate. The test chambers were glass aquariums (25X46X25 cm) containing approximately 20 liters of medium to a depth of 19 cm. Supplementary aeration was not provided. A photoperiod of 16 hours light: 8 hours dark was

maintained and daily records of temperature, pH and dissolved oxygen were kept for each control and test vessel. The fish were not fed during the 96-hour exposure period.

Fish per Vessel: 10 fish in each vessel.

Dose Selection: Nominal: 0.25, 0.55, 1.2, 2.7 and 5.8 mg/L. Mean measured concentrations: 0.25, 0.53, 1.2, 2.6 and 4.9 mg/L

Renewal and Exposure: Fish were exposed to the test or control conditions for a period of 96 hours under continuous flow conditions.

Temperature range: Treatment and controls groups were maintained at 23±1°C throughout the exposure period.

Analytical Results

Occasion	Nominal Concentration (mg/L)	Measured Concentration (mg/L)	Recovery as a % of nominal
0 Hours	Solvent Control R1	ND	-
	Solvent Control R2	ND	-
	0.25 R1	0.2379	95.2
	0.25 R2	0.2635	105
	0.55 R1	0.4826	87.7
	0.55 R2	0.5002	90.9
	1.2 R1	1/218	102
	1.2 R2	1.219	102
	2.7 R1	2.549	94.4
	2.7 R2	3.093	115
	5.8 R1	4.489	77.4
	5.8 R2	4.850	83.6
24 Hours	Solvent Control R1	ND	-
	Solvent Control R2	ND	-
	0.25 R1	0.2586	103
	0.25 R2	0.2461	98.4
	0.55 R1	0.4910	89.3
	0.55 R2	0.5442	98.9
	1.2 R1	1.131	94.2
	1.2 R2	1.156	96.3
	2.7 R1	2.661	98.6
	2.7 R2	2.803	104
	5.8 R1	5.076	87.5
	5.8 R2	5.633	97.1
48 Hours	Solvent Control R1	ND	-
	Solvent Control R2	ND	-
	0.25 R1	0.2639	106
	0.25 R2	0.2627	105
	0.55 R1	0.5095	92.6
	0.55 R2	0.5546	101
	1.2 R1	1.132	94.3
	1.2 R2	1.149	95.8
	2.7 R1	2.437[#]	90.3
	2.7 R2	2.232	82.7
	5.8 R1	4.824	83.2
	5.8 R2	4.566	78.7

72 Hours	Solvent Control R1	ND	-
	Solvent Control R2	ND	-
	0.25 R1	0.2622	105
	0.25 R2	0.2306	92.2
	0.55 R1	0.5678	103
	0.55 R2	0.6571	119
	1.2 R1	1.417	118
	1.2 R2	1.250	104
	2.7 R1	2.658	98.4
	2.7 R2	2.694	99.8
	5.8 R1	5.239	90.3
	5.8 R2	4.897	84.4
96 Hours	Solvent Control R1	ND	-
	Solvent Control R2	ND	-
	0.25 R1	0.2320	92.8
	0.25 R2	0.2366	94.6
	0.55 R1	0.4695	85.4
	0.55 R2	0.5201	94.6
	1.2 R1	1.150	95.9
	1.2 R2	1.139	94.9
	2.7 R1	2.390	88.5
	2.7 R2	2.696	99.9
	5.8 R1	4.428	76.4
	5.8 R2	1.659*	28.6

ND – None detected (limit of detection: 0.007 mg/L)
* - Anomalous result excluded from study. The result of analysis of the duplicate sample was low (12.5%), probably due to biodegradation during storage.
# - Result from analysis of the duplicate sample. The original result was low (53.0%) and thought to be due to sampling or analysis error.
R1 and R2 – Replicate Number.

Results

Time (hours)	**LC_{50} (mg/L)**	**95% confidence limits (mg/L)**
0.25-48	> 4.9	-
72	4.7	4.0 - >4.9
96	3.7	3.2 – 4.3

Highest test concentration resulting in 0% mortality: 1.2 mg/L
Lowest test concentration resulting in 100% mortality: >4.9 mg/L
No-observed effect concentration: 1.2 mg/L

Conclusions

Remarks:
Results of additional studies show that neither the dipropylene glycol dibenzoate nor its degradates are persistent in natural water and therefore are not likely to present any potential long-term danger to aquatic organisms.

Degradation of dipropylene glycol dibenzoate in river water was established after a lag period of approximately 13 hours after which removal of dipropylene glycol dibenzoate was rapid and complete by

46 hours when levels were below the limit of quantitation of the analytical method (0.0054 mg/L). Once degradation was established, the overall zero order rate constant for degradation, calculated from a linear regression of concentration against time between, 13 and 31 hours was 0.00655 h^{-1}; the half life (t1/2) of dipropylene glycol dibenzoate in river water was 11.5 hours. The degradate dipropylene glycol monobenzoate was detected in river water samples at 13.17, 21, 25.5 and 31 hours (range 0.0124 to 0.0415 mg/L); benzoic acid, which was detected at intervals during the test and ranged in concentration from 0.00555 mg/l one hour after mixture preparation to 0.00696 mg/L after 31 hours. The results of this test showed that following dissolution, dipropylene glycol dibenzoate is readily biodegraded in river water with a half-life of 11.5 hours. None of the degradates likely to be formed as a result of breakdown of the dipropylene glycol dibenzoate (dipropylene glycol monobenzoate or benzoic acid) were detected at significant levels in test media which suggested that these were not persistent.

Data Quality 1, Reliable without restrictions

References

Dipropylene Glycol Dibenzoate. Acute Toxicity for Fathead Minnow (*Pimephales promelas*). Huntingdon Life Sciences. 2000.

Dipropylene Glycol Dibenzoate. Preliminary Assessment of its Degradation in Laboratory and Natural Waters. Huntingdon Life Sciences. 1999.

Benzoflex 2-45 (Diethylene Glycol Dibenzoate) Benzoflex 9-88 (Dipropylene Glycol Dibenzoate) Benzoflex S-358 (Triethylene Glycol Dibenzoate). Ecotoxicological Properties and WGK Classification. Huntingdon Life Sciences. 2000.

Other aquatic toxicity tests:

Dipropylene Glycol Dibenzoate. Acute Toxicity For Rainbow Trout (*Oncorhynchus mykiss)*. Huntingdon life Sciences. 2000. Study was conducted in accordance with OECD 203 "Fish, Acute Toxicity Test.
Under dynamic exposure conditions, the 0.25 – 96 hr LC_{50} is > 3.0 mg/L
Highest concentration tested resulting in 0% mortality: 3.0 mg/L
Lowest concentration tested resulting in 100% mortality: >3.0 mg/L
No-observed effect concentration: 3.0 mg/L
Results expressed in terms of mean measured concentration.

Acute Toxicity to Aquatic Invertebrates

Test Substance: See boiling point for purity

Method

Guideline	OECD 202 Part 1
Test Type	*Daphnia* Acute Immobilization Test
GLP	Yes
Year	1997
Species/Strain	*Daphnia magna*
Analytical Monitoring	Determination of test material concentration at 0 and 48 hours
Exposure Period	48 hours
Test Details	Static without renewal
Statistical Methods	EL_{50} and 95% confidence limits were calculated using the Thompson and Weil model (Thompson WR and Weil CS, 1952, Biometrics **8**: 51-54).

Test Condition Remarks

Age at Initiation: Less than 24 hours

Test Conditions: As specified in OECD 202 following advice given in ECETOC 1996 Monograph No. 26.

Solvent: Reconstituted medium Elendt M4.

Vessel: 250 ml capacity glass jars containing 200 ml of prepared test medium. The jars were loosely covered to minimize evaporative losses.

Daphnids per Vessel: 5

Dose Selection: Nominal initial loading rate: 1.0, 2.2, 4.6, 10, 22, 46 and 100 mg/L.

Temperature range: 19.3 – 19.7 °C
Solution pH range: 7.9-8.1

Dissolved Oxygen: 7.0-8.0

Analytical Results

Occasion	Nominal Initial Loading(mg/L)	% of Loading Monobenzoate (mg/L)	% of Loading Dibenzoate (mg/L)
0 Hours (Fresh)	Control	ND	ND
	1.0	3.6	33.9
	2.2	5.0	40.7
	4.6	5.2	30.8
	10	4.8	49.4
	22	5.8	40.3
	46	7.6	13.8
	100	13.9	19.3

48 Hours (expired)	Control 1.0 2.2 4.6 10 22 46 100	ND 9.0 13.7 16.0 18.2 11.3 8.3 6.9	ND 18.1 27.3 26.8 26.6 27.5 9.2 7.1

Results

Time (hours)	**EL_{50} (mg/L)**	**95% confidence limits (mg/L)**
24	43.2	25.9-72.1
48	19.31	13.1-28.5

Highest initial loading rate resulting in 0% mortality: 2.2 mg/L*
Lowest initial loading rate resulting in 100% mortality: > 100 mg/L
No-observed effect loading rate: 2.2 mg/L*

*5% immobilization was observed in the 2.2 mg/L test group. This level of immobilization is not considered significant.

Nominal Initial Loading Rate (mg/L)	Cumulative immobilized *Daphnia magna* (initial population 5/replicate) 24 hours						Cumulative immobilized *Daphnia magna* (initial population 5/replicate) 48 hours					
	R1	R2	R3	R4	Total	%	R1	R2	R3	R4	Total	%
Control	0	0	0	1	1	5	1	0	0	1	2	10
1.0	0	1	0	0	1	5	0	1	1	0	2	10
2.2	0	0	0	0	0	0	0	0	0	1	1	5
4.6	0	1	0	0	1	5	0	1	3	1	5	25
10	1	0	0	0	1	5	2	3	1	2	8	40
22	1	3	4	3	11	55	4	5	4	4	17	85
46	4	3	2	2	11	55	4	3	3	2	12	60
100	4	3	4	3	14	70	5	4	5	4	18	90

R1 – Replicate 1
R2 – Replicate 2
R3 – Replicate 3
R4 – Replicate 4

Conclusions

Remarks: The 48 hour EL50 was determined to be 19.3 mg/l. The No Observed Effect Loading rate was 2.2 mg/l.

Results of additional studies show that neither the dipropylene glycol dibenzoate nor its degradates are persistent in natural water and therefore are not likely to present any potential long-term danger to aquatic organisms.

Degradation of dipropylene glycol dibenzoate in river water was established after a lag period of approximately 13 hours after which removal of dipropylene glycol dibenzoate was rapid and complete by 46 hours when levels were below the limit of quantitation of the analytical method (0.0054 mg/L). Once degradation was established, the overall zero order rate constant for degradation, calculated from a linear regression of concentration against time, between 13 and 31 hours was 0.00655 h^{-1}; the half life (t1/2) of dipropylene glycol dibenzoate in river water was 11.5 hours. The degradate dipropylene glycol monobenzoate was detected in river water samples at 13.17, 21, 25.5 and 31 hours (range 0.0124 to 0.0415 mg/L); benzoic acid, which was detected at intervals during the test and ranged in concentration from 0.00555 mg/l one hour after mixture preparation to 0.00696 mg/L after 31 hours. The results of this test showed that following dissolution, dipropylene glycol dibenzoate is readily biodegraded in river water with a half-life of 11.5 hours. None of the degradates likely to be formed as a result of breakdown of the dipropylene glycol dibenzoate (dipropylene glycol monobenzoate or benzoic acid) were detected at significant levels in test media which suggested that these were not persistent.

Data Quality 1, Reliable without restrictions

References

Dipropylene Glycol Dibenzoate. Acute Toxicity to *Daphnia magna*. Huntingdon Life Sciences. 2001.

Dipropylene Glycol Dibenzoate. Preliminary Assessment of its Degradation in Laboratory and Natural Waters. Huntingdon Life Sciences. 1999.

Benzoflex 2-45 (Diethylene Glycol Dibenzoate) Benzoflex 9-88 (Dipropylene Glycol Dibenzoate) Benzoflex S-358 (Triethylene Glycol Dibenzoate). Ecotoxicological Properties and WGK Classification. Huntingdon Life Sciences. 2000.

TOXICITY TO AQUATIC PLANTS

Test Substance See boiling point for purity

Test Method OECD 201

GLP: Yes

Date: 1997
Species *Selenastrum capricornutum,* Strain number CCAP 278/4
Element basis Area under the curve (72 hours, 96 hours), growth rate (0-72 hours, 0-96 hours)
Exposure period 96 hours (November 7-November 11, 1997)
Test organisms
Sterile nutrient medium was inoculated from a master culture and cultured under continuous illumination (~7000 lux) in an orbital incubator at 22°C, to give an algal suspension in log phase growth characterized by a cell density of 1.4 $x10^7$ cells/ml.

Test Conditions
Test Temperature Range 24 ± 1°C
Exposure vessel Type
250 ml conical flask each containing 100 ml of test or control culture was loosely stoppered and placed in a Gallenkamp Illuminated Orbital Incubator.
Light levels and quality during exposure
Incubated, without medium renewal for 96 hours under continuous illumination of approximately 7000 lux provided by 7X30 W "universal white" 1 meter fluorescent tubes.

Test Design
Number of replicates Seven exposure levels were prepared plus one untreated control, each in triplicate
Nominal Initial Loading Rates 0.10, 0.22, 0.46, 1.0, 2.2, 4.6, and 10 mg/l

Results	**NOEL**	**EL_{10}**	**EL_{50}**	**EL_{90}**
Area Under curve (72 hours)	0.22	0.15	1.1	7.9
Growth Rate (0-72 hours)	1.0	0.89	4.9	27
Area Under Curve (96 hours)	*	0.19	0.95	4.8
Growth Rate (0-96 hours)	0.46	0.87	3.6	15

*A NOEL was not observed for this data set as an effect was observed at the lowest loading rate tested

Mean cell density of control at 0 hour 1.48 x 10^4 cells/ml
Mean cell density of control at 96 hour 1.77 x 10^6 cells/ml

Chemical analyses of the two main components in fresh and expired water samples were carried out. The measured concentration of dipropylene glycol dibenzoate in fresh samples was between 69 and 99% of the total loading rate. For dipropylene glycol monobenzoate, the mean measured concentration in fresh samples was between 5 and 6% of the total loading rate. In expired samples the amount of dibenzoate decreased to between 41 and 66% of initial loading rates in samples from vessels containing algae. At the same time, measured concentrations of the monobenzoate had increased to between 7.8 and 24% of the initial loading rates in expired samples suggesting this component was a degradation product of the dibenzoate. Analysis of the expired samples from the 1.0 mg/l initial loading rate that were held in test conditions but without algae showed that concentrations of both measured components had not changed from the levels found in fresh samples. This suggested that the presence of algal cells was facilitating the degradation the dibenzoate.

Results of additional studies show that neither the dipropylene glycol dibenzoate nor its degradates are persistent in natural water and therefore are not likely to present any potential long-term danger to aquatic organisms.

Degradation of dipropylene glycol dibenzoate in river water was established after a lag period of approximately 13 hours after which removal of dipropylene glycol dibenzoate was rapid and complete by 46 hours when levels were below the limit of quantitation of the analytical method (0.0054 mg/L). Once degradation was established, the overall zero order rate constant for degradation, calculated from a linear regression of concentration against time, between 13 and 31 hours was 0.00655 h^{-1}; the half life (t1/2) of dipropylene glycol dibenzoate in river water was 11.5 hours. The degradate dipropylene glycol monobenzoate was detected in river water samples at 13.17, 21, 25.5 and 31 hours (range 0.0124 to 0.0415 mg/L); benzoic acid, which was detected at intervals during the test and ranged in concentration from 0.00555 mg/l one hour after mixture preparation to 0.00696 mg/L after 31 hours. The results of this test showed that following dissolution, dipropylene glycol dibenzoate is readily biodegraded in river water with a half-life of 11.5 hours. None of the degradates likely to be formed as a result of breakdown of the dipropylene glycol dibenzoate (dipropylene glycol monobenzoate or benzoic acid) were detected at significant levels in test media which suggested that these were not persistent.

Data Quality 1, Reliable without restrictions

References

Benzoflex 9-88. Algal Growth Inhibition. Huntingdon Life Sciences. 2001

Dipropylene Glycol Dibenzoate. Preliminary Assessment of its Degradation in Laboratory and Natural Waters. Huntingdon Life Sciences. 1999.

Benzoflex 2-45 (Diethylene Glycol Dibenzoate) Benzoflex 988 (Dipropylene Glycol Dibenzoate) Benzoflex S-358 (Triethylene Glycol Dibenzoate). Ecotoxicological Properties and WGK Classification. Huntingdon Life Sciences. 2000.

ENVIRONMENTAL FATE

PHOTODEGRADATION

Dipropylene Glycol Dibenzoate is structurally similar to diethylene glycol dibenzoate and triethylene glycol dibenzoate. They contain the same functional groups – aromatic rings, ester groups ether linkages and hydroxyl groups. Given that the same functional groups are present, the chemical properties of each substance can be expected to be similar with any difference being attributable to molecular weight.

As the molecular weight of dipropylene glycol dibenzoate (342.18) is between those of diethylene glycol dibenzoate (314.34) and triethylene glycol dibenzoate (358.39), its physical and chemical properties are similarly likely to be the two extremes. It should therefore be possible to use data from the higher and lower molecular weight dibenzoate analogues in assessing the properties of the intermediate molecular weight substance, dipropylene glycol dibenzoate.

Diethylene Glycol Dibenzoate

The rate constant and half-life for the atmospheric gas-phase reaction between photolytically produced hydroxyl radicals and diethylene glycol dibenzoate have been estimated using the software AOPWIN v1.88.

The rate constant for the reaction, assuming a 24-hour day and a hydroxyl radical concentration of 1.5 x 10^6 mol/cm^3 was18.95 x 10^{-12} cm^3/mol-sec and the half-life was 0.282 days (6.772 hours).

The program did not estimate a rate constant for reaction with ozone (only olefins and acetylenes are estimated) and there were no structures matched with the experimental database.

Triethylene Glycol Dibenzoate

The rate constant and half-life for the atmospheric gas-phase reaction between photolytically produced hydroxyl radicals and triethylene glycol dibenzoate have been estimated using the software AOPWIN v1.88.

The rate constant for the reaction, assuming a 24-hour day and a hydroxyl radical concentration of 1.5 x 10^6 mol/cm^3 was 32.97 x 10^{-12} cm^3/mol-sec and the half-life was 0.162 days (3.893 hours).

The program did not estimate a rate constant for reaction with ozone (only olefins and acetylenes are estimated) and there were no structures matched with the experimental database.

Reliability: Estimated value based on accepted model.

Reference: Diethylene Glycol Dibenzoate. Estimation of Photodegradation Using the Atmospheric Oxidation Program (AOPWIN). Huntingdon Life Sciences. 2001.

Triethylene Glycol Dibenzoate. Estimation of Photodegradation Using the Atmospheric Oxidation Program (AOPWIN). Huntingdon Life Sciences. 2001.

Other Studies: Butz RG, Atallah YH, Yu CC and Calo CJ. Environmental Toxicology and Chemistry. 1, 337. 1982. Benzoflex 9-88 spotted on silica gel has been shown to photodegrade directly under the influence of UV light with a half-life of 12.8 days.

STABILITY IN WATER

Dipropylene Glycol Dibenzoate is structurally similar to diethylene glycol dibenzoate and triethylene glycol dibenzoate. They contain the same functional groups – aromatic rings, ester groups ether linkages and hydroxyl groups. Given that the same functional groups are present, the chemical properties of each substance can be expected to be similar with any difference being attributable to molecular weight.

As the molecular weight of dipropylene glycol dibenzoate (342.18) is between those of diethylene glycol dibenzoate (314.34) and triethylene glycol dibenzoate (358.39) its physical and chemical properties are similarly likely to be the two extremes. It should therefore be possible to use data from the higher and lower molecular weight dibenzoate analogues in assessing the properties of the intermediate molecular weight substance, dipropylene glycol dibenzoate.

Diethylene Glycol Dibenzoate

The aqueous hydrolysis rate constant and half-life for diethylene glycol dibenzoate have been estimated using the software program HYDROWIN v1.66.

The calculated rate constant for the base-catalyzed reaction (K_b) at pH>8 and at 25°C was estimated to be 1.645×10^{-1} L/mol-sec. Half-lives at pH 7 and 8 were estimated to be 1.335 years and 48.77 days, respectively.

The program does not calculate neutral hydrolysis rate constants. Therefore, reported half-lives may be over estimates.

The fragment $–CH_2- CH_2-O-R$ was not available in the program's library. Therefore, fragment $- CH_2- CH_2 – 0-CH_3$ was substituted. This is unlikely to have affected the estimation result significantly.

Triethylene Glycol Dibenzoate

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The fragment $–CH_2- CH_2-O-R$ was not available in the program's library. Therefore fragment $- CH_2- CH_2 – 0-CH_3$ was substituted. This is unlikely to have affected the estimation result significantly.

Reliability: Estimated value based on accepted model.

References: Diethylene Glycol Dibenzoate. Estimation of Hydrolysis Rate Using the HYDROWIN Program. Huntingdon Life Sciences. 2001.

Triethylene Glycol Dibenzoate. Estimation of Hydrolysis Rate Using the HYDROWIN Program. Huntingdon Life Sciences. 2001.

Transport (Fugacity)

Dipropylene Glycol Dibenzoate is structurally similar to diethylene glycol dibenzoate and triethylene glycol dibenzoate. They contain the same functional groups – aromatic rings, ester groups ether linkages and hydroxyl groups. Given that the same functional groups are present, the chemical properties of each substance can be expected to be similar with any difference being attributable to molecular weight.

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Diethylene Glycol Dibenzoate

The fate and behavior of diethylene glycol dibenzoate in a model environment consisting of four main components, air, water, soil and sediment, has been evaluated using the Mackay Level III Fugacity Model, version 2.10.

Inputs:

Chemical Type	1	A chemical that partitions into all media
Molecular mass	314.34	Molecular formula $C_{18}H_{18}O_5$
Data Temperature	25°C	
Log K_{ow}	3.2	VCL261/972408
Water Solubility(g/m^3)	38.3	VCL261/972408
Vapor Pressure (Pa)	1.73E-05	VCL261/972408
Melting Point	24°C	VCL 261/972408
Half-life in Air	6.772 hours	VCL363/010076
Half-life in Water	62.5 hours	VCL298/983319 A factor of ten was added for this study to allow for probability of lower degradation in some other water bodies
Half-life in Soil	125 hours	Assumed twice that of water. This is in line with advice in the TGD Part II for a readily biodegradable substance with a soil-water partition coefficient of less than 100 l/kg
Half-life in Bulk Sediment	125 hours	Assumed twice that of water. This is in line with advice in the TGD Part II for a readily biodegradable substance with a soil-water partition coefficient of less than 100 l/kg
Half-life in suspended Sediment	62.5 hours	No data therefore because the close proximity of the bulk water phase the half-life for the latter was used.
Half-life in Fish	62.5 hours	No data therefore because the close proximity of the bulk water phase the half-life for the latter was used.
Half-life in Aerosol	6.772 hours	No data therefore because the close proximity of the bulk air phase the half-life for the latter was used.

The parameters that define the model environment are:
Volume of each environmental compartment (m^3)
Density of each environmental compartment (kg/m^3)

Organic carbon content of soil and sediments (g/g)
Lipid content (kg/m^3)
Transport Velocities between compartments (m/h)

The route and magnitude of emissions to the environment will vary depending on the stage of the product life cycle being considered. Because no information was available on probable real-life emissions into the environment the recommendation of Mackay et al (1996) was followed; that is the model was run for 1000 kg/h emissions to each of the air, water and soil compartments individually and then in total. This standardized approach allows comparison with other compounds and provides information on the main source of the chemicals in each compartment. Specific properties which should be taken into account when evaluating outputs from the model are that it has a very low vapor pressure so that losses to the atmosphere during manufacture and processing will be limited and that its ready biodegradability means that concentrations in the effluent form sewage treatment plants will be very low.

Under equilibrium steady state condition (Fugacity Model Level 1) diethylene glycol dibenzoate distributed almost entirely between the soil and water compartments

Compartment	Amount
Air	0.0012%
Soil	57.62%
Water	41.05%
Sediment	1.28%

Using the Level III program and with emissions of 1000 kg/h to each of the air, water and soil compartments, the model estimated the following distribution:

Compartment	Amount
Air	0.73%
Soil	76.1%
Water	23.2%
Sediment	0.040%

The predominant routes of loss were by degradation in the soil, water and air compartment (53.8, 32.8 and 9.50% respectively) and advection from the water compartment (2.96%). The estimated mean residence time for diethylene glycol dibenzoate in the model environment (persistence) was 128 hours.

Reliability: Estimated values based on accepted model.

References:

Diethylene Glycol Dibenzoate. Estimation of Environmental Fate Using the Mackay Level II Fugacity Model. Huntingdon Life Sciences. 2001.

VCL 261 - Benzoflex 9-88. Determination of Physico-Chemical Properties. Huntingdon Life Sciences. 1999

VCL363 – Diethylene Glycol Dibenzoate. Estimation of Photodegradation Using the Atmospheric Oxidation Program (AOPWIN). Huntingdon Life Sciences. 2001.

VCL 298 – Diethylene Glycol Dibenzoate. Preliminary Assessment of its Degradation in Laboratory and Natural Waters. Huntingdon Life Sciences. 1999.

Technical Guidance Document (TGD) in Support of Commission Directive 93/67/EEC on Risk Assessment for New Notified Substances and Commission regulation (EC) No. 1488/94 on Risk Assessment for Existing Substances Part II, pp. 282-285.

Mackay, D., DiGuardo, A.., Paterson, S. and Cowan C. (1996) Evaluating the Environmental Fate of a Variety of Types of Chemicals Using the EQC Model. Environ Toxicol Chem, **15**, p. 1627.

Triethylene Glycol Dibenzoate

The fate and behavior of triethylene glycol dibenzoate in a model environment consisting of four main components, air, water, soil and sediment, has been evaluated using the Mackay Level III Fugacity Model, version 2.10.

Inputs:

Chemical Type	1	A chemical that partitions into all media
Molecular mass	358.39	Molecular formula $C_{20}H_{22}O_6$
Data Temperature	25°C	
Log K_{ow}	3.2	VCL273/972409
Water Solubility(g/m^3)	30.4	VCL273/972409
Vapor Pressure (Pa)	2.533E-05	VCL273/972409
Melting Point	46.25°C	VCL273/972409
Half-life in Air	3.893 hours	VCL366/010079
Half-life in Water	280 hours	VCL297/983318 A factor of ten was added for this study to allow for probability of lower degradation in some other water bodies
Half-life in Soil	560 hours	Assumed twice that of water. This is in line with advice in the TGD Part II for a readily biodegradable substance with a soil-water partition coefficient of less than 100 l/kg
Half-life in Bulk Sediment	560 hours	Assumed twice that of water. This is in line with advice in the TGD Part II for a readily biodegradable substance with a soil-water partition coefficient of less than 100 l/kg
Half-life in suspended Sediment	280 hours	No data therefore because the close proximity of the bulk water phase the half-life for the latter was used.
Half-life in Fish	280 hours	No data therefore because the close proximity of the bulk water phase the half-life for the latter was used.
Half-life in Aerosol	3.893 hours	No data therefore because the close proximity of the bulk air phase the half-life for the latter was used.

The parameters that define the model environment are:
Volume of each environmental compartment (m^3)
Density of each environmental compartment (kg/m^3)
Organic carbon content of soil and sediments (g/g)
Lipid content (kg/m^3)
Transport Velocities between compartments (m/h)

The route and magnitude of emissions to the environment will vary depending on the stage of the product life cycle being considered. Because no information was available on probable real-life emissions into the environment the recommendation of Mackay et al (1996) was followed; that is the model was run for 1000 kg/h emissions to each of the air, water and soil compartments individually and then in total. This standardized approach allows comparison with other compounds and provides information on the main source of the chemicals in each compartment. Specific properties which should be taken into account

when evaluating outputs from the model are that it has a very low vapor pressure so that losses to the atmosphere during manufacture and processing will be limited and that its ready biodegradability means that concentrations in the effluent form sewage treatment plants will be very low.

Under equilibrium steady state condition (Fugacity Model Level 1) triethylene glycol dibenzoate distributed almost entirely between the soil and water compartments

Compartment	Amount
Air	0.0025%
Soil	57.62%
Water	41.05%
Sediment	1.28%

Using the Level III program and with emissions of 1000 kg/h to each of the air, water and soil compartments, the model estimated the following distribution:

Compartment	Amount
Air	0.16%
Soil	79.3%
Water	20.4%
Sediment	0.14%

The predominant routes of loss were by reaction in the soil, water and air compartment (49.4, 25.4 and 14.0% respectively) and advection from the water compartment (10.3%). The estimated mean residence time for triethylene glycol dibenzoate in the model environment (persistence) was 503 hours.

Reliability: Estimated values based on accepted model.

References:

Triethylene Glycol Dibenzoate. Estimation of Environmental Fate Using the Mackay Level II Fugacity Model. Huntingdon Life Sciences. 2001.

VCL 273 - Benzoflex 2-45. Determination of Physico-Chemical Properties. Huntingdon Life Sciences. 1999

VCL366 – Triethylene Glycol Dibenzoate. Estimation of Photodegradation Using the Atmospheric Oxidation Program (AOPWIN). Huntingdon Life Sciences. 2001.

VCL 297 – Triethylene Glycol Dibenzoate. Preliminary Assessment of its Degradation in Laboratory and Natural Waters. Huntingdon Life Sciences. 1999.

Technical Guidance Document (TGD) in Support of Commission Directive 93/67/EEC on Risk Assessment for New Notified Substances and Commission regulation (EC) No. 1488/94 on Risk Assessment for Existing Substances Part II, pp. 282-285.

Mackay, D., DiGuardo, A.., Paterson, S. and Cowan C. (1996) Evaluating the Environmental Fate of a Variety of Types of Chemicals Using the EQC Model. Environ Toxicol Chem, **15**, p. 1627.

ACUTE TOXICTY

ORAL

Test Substance: See boiling point for purity.

Test Method: OECD 401

GLP: Yes
Date: 1998
Laboratory: Huntingdon Life Sciences
Species/Strain: Rat, Sprague-Dawley
Sex: Male and Female

Results: Acute Oral LD_{50} and 95% confidence limits
Male: 5072 (4455-5774) mg/kg
Female: 3295 (2857-3801) mg/kg
Combined: 3914 (2957-4844) mg/kg

Number of Deaths at Each Dose Level:

3200 mg/kg:	2 females
5000 mg/kg:	2 males and 5 females
6400 mg/kg:	5 males

Time of Death of Each Animal:

Day 2

3200 mg/kg:	1 females
5000 mg/kg:	1 male and 4 females
6400 mg/kg:	5 males

Day 3

3200 mg/kg:	1 female
5000 mg/kg:	1 male and 1 female

Description of Clinical Effects:

Piloerection was observed in all rats within 15 minutes of dosing. This sign persisted and was accompanied in rats later during the study by:

Hunched posture, waddling/unsteady gait, lethargy and pallor of the extremities in all rats;

Partially closed eyelids in three males at 2000 mg/kg, four females at 3200 mg/kg, in all rats at 5000 mg/kg and in all males at 6400 mg/kg

Increased salivation in all males at 2000 mg/kg, one female at 3200 mg/kg, all rats at 5000 mg/kg and three males at 6400 mg/kg

Walking on toes in all rats at 2000 and 5000 mg/kg and four females at 3200 mg/kg;

Ungroomed appearance in all males at 2000 and 6400 mg/kg, four females at 3200 mg/kg and in all rats at 5000 mg/kg

Respiratory distress (characterized by increased or decreased respiration) in all males at 2000 mg/kg, four females at 3200 mg/kg and in all rats at 5000 mg/kg

Soft to liquid feces in one male at 2000 mg/kg

Clonic convulsions in three males at 5000 mg/kg

Increased lacrimation and body tremors in three females at 3200 mg/kg and all rats at 5000 mg/kg;

Cold body surfaces in three males and all females at 2000 mg/kg, four females at 3200 mg/kg and all rats at 5000 mg/kg;

Prostration in one female at 3200 mg/kg and two males and two females at 5000 mg/kg

Red brown stains around the muzzle in four females at 3200 mg/kg and two males and one female at 5000 mg/kg

Red brown stains around the urogenital area in three females at 3200 mg/kg one male at 5000 mg/kg;

Sensitivity to handling in four females at 3200 mg/kg and two males at 5000 mg/kg

Aggressive behavior to cagemates in three females at 3200 mg/kg

Brown staining on dorsal area in three females at 3200 mg/kg

Recovery of surviving rats was complete, with the exception of piloerection, by either Day 4 (females 2000 mg/kg), Day 5 (males 2000 mg/kg), Day 8 (males 5000 mg/kg) or Day 15 (females 3200 mg/kg).

Necropsy findings:

Females at 3200 mg/kg

Congestion (characterized by dark appearance/prominent blood vessels/inflammation) in brain, heart, lungs, liver, spleen and kidneys. Fluid content and gaseous distension was also seen in pale stomach and along the alimentary tract.

Males and Females 5000 mg/kg

Congestion (characterized by dark appearance/prominent blood vessels/inflammation) in subcutaneous tissue, brain, heart, lungs, liver, spleen and kidneys. Fluid content and gaseous distension was also seen in stomach and along the alimentary tract.

Males 6400 mg/kg

Congestion (characterized by dark appearance/prominent blood vessels/inflammation) in subcutaneous tissue, brain, heart, lungs, liver, spleen and kidneys. Fluid content and gaseous distension was also seen in pale stomach and along the alimentary tract. In addition, a white thickened deposit was noted in the urinary bladder.

Data Quality: 1, Reliable without restrictions

References: Benzoflex 9-88 Acute Oral LD_{50}. Huntingdon Life Sciences. 1998

Other studies: International Research and Development Corporation (IRDC). 1975. Oral LD_{50} for rats and mice is in the range of 4,000 to 5,500 mg/kg.

DERMAL

Test Substance: See boiling point for purity.

Test Method: OECD 402

GLP: Yes
Date: 1997
Laboratory: Huntingdon Life Sciences
Species/Strain: Rat, Sprague-Dawley
Sex: Male and Female

Results: Acute Dermal LD_{50} >2000 mg/kg

Number of Deaths at Each Dose Level: No deaths

Description of Clinical Effects: No signs of systemic reaction to treatment were observed in any animal throughout the observation period.

Data Quality: 1, Reliable without restrictions

References: Benzoflex 9-88. Acute Dermal LD_{50}. Huntingdon Life Sciences. 1998

AMES MUTAGENICITY

Test Substance: See boiling point for purity.

Solvent: DMSO

Study Type: Bacterial reverse mutation assay

Test Method: OECD 471, 472

GLP: Yes

Date: 1997
Laboratory: Huntingdon Life Sciences
Species/Strain: S. typhimurium: TA 1535 his G46 rfa uvrB
TA 1537 his C3076 rfa uvrB
TA 98 his D3052 rfa uvrB pKM101
TA 100 his G46 rfa uvrB pKM101

E. coli CM891 WP2 trp uvrA pKM101

Concentrations: 5000, 1500, 500, 150, 50, 15, 5 µg/plate
Metabolic Activation: Sprague-Dawley rat liver
Quantity of Activator: 0.5 ml
Induction: Stimulated by Aroclor 1254

Criteria for Evaluating Results:

(a) If treatment with test substance produces an increase in reverent colony numbers of at least 2 times the current solvent controls with some evidence of a positive dos-relationship, in a specific bacterial strain reproduced with or without S9 mix, it is considered to show evidence of mutagenic activity in this test system. No statistical analysis is performed.
(b) If treatment with a test substance does not produce reproducible increases of at least 1.5 times the current solvent controls, at any dose level, with any bacterial strain, it is considered to show evidence of mutagenic activity in this test system. No statistical analysis is performed.
(c) If the results obtained fail to satisfy the criteria for a clear "positive" or "negative" response given in paragraphs (a) and (b), additional testing may be performed in order to resolve the issue of the substance's mutagenic activity in this test system. Modifications to the experimental method will usually be considered, such as the use of a narrower dose range and different levels of S9 mix. Should an increase in reverent colony numbers then be observed which satisfies paragraph (a) the substance is considered to show evidence of mutagenic activity in this test system. If no clear "positive" response can be obtained, the test data may be subjected to analysis to determine the statistical significance of any observed increases in reverent colony numbers. The statistical procedures used will be those described by Mahon *et al* (1989) and will usually be analysis of variance followed by Dunnett's test.

Mahon, G.A.T., Green, M.H.L., Middleton, B., Mitchell, I de G., Robinson, W.D. and Tweats, D.J. (1989). Analysis of data from microbial colony assay in: Kirkland, D.J. (ed.) *UKEMS Subcommittee on Guidelines for Mutagenicity Testing. Report Part III. Statistical Evaluation of Mutagenicity Data.* P.26. Cambridge University Press. Cambridge.

Positive/Negative Controls: Positive without S9:
N-Ethyl-N'-nitro-N-nitrosoguanidine in DMSO @ 5µg/plate for TA 1535, 3µg/plate for TA 100 and 2µg/plate for CM891

9-Aminoacridine in DMSO @ 1µg/plate for TA 1537

2-Nitrofluorene in DMSO @ 1µg/plate for TA 98

Positive with S9

2-Aminoanthracene in DMSO @ 2µg/plate for TA 1535 and 10µg/plate for CM891

Benzo[a] pyrene in DMSO @ 5µg/plate TA 1537, TA 98 and TA 100

Negative
DMSO

Repeat Test: Second test includes a preincubation stage. First test is only the Standard Plate Incorporation.

Results:

Cytotoxic Concentrations: With and Without Metabolic Activation –
5000 and 1500 µg/plate. Observed in the second preincubation assay.

Precipitation Concentration: 5 mg/plate – cloudy solution with a few very small observable globules floating on the surface.
1.5 mg/plate – cloudy solution but no observable globules
0.5 mg/plate – slightly cloudy solution
0.15 mg/plate – no observable cloudiness

Genotoxic Effects: No effects observed with or without metabolic activation. No evidence of mutagenicity in this bacterial system.

Data Quality: 1, Reliable without restrictions

References: Benzoflex 9-88. Bacterial Mutation Assay (*S.typhimurium* and *E. coli*). Huntingdon Life Sciences. 1998

Other Studies: Litton Bionetics (LB). 1978. Benzoflex 9-88 was not mutagenic in bacteria or yeast.

MAMMALIAN CELL MUTATION

Test Substance: See boiling point for purity.

Solvent: DMSO
Study Type: Nonbacterial mammalian cell gene mutation assay
Test Method: OECD 476
GLP: Yes
Date: 1997
Laboratory: Huntingdon Life Sciences
Species/Strain: Mouse lymphoma L5178Y
Concentrations: 150,200,225,250 µg/ml
Metabolic Activation: Sprague-Dawley rat liver
Quantity of Activator: 4 ml
Induction: Stimulated by Aroclor 1254

Criteria for Evaluating Results:

Criteria for a positive response:

An increase of at least 100 in the mutant frequency in treated cultures relative to the concurrent control.

The demonstration of a statistically significant increase in mutant frequency following treatment with the test substance.

Evidence of a dose relationship over at least two consecutive dose levels, in any increases in mutation frequency.

Demonstration of reproducibility in any increase in mutant frequency.

An increase in absolute colony numbers in the treated cultures.
The RTG of cultures showing an increase in mutant frequency should not be less than 10%.

Positive/Negative Controls: Positive without S9:
Methylmethane sulphonate in DMSO
Positive with S9:
20-methylcholanthrene in DMSO

Results:

Genotoxic Effects: No effects observed with or without metabolic activation. No evidence of mutagenicity in this *in vitro* gene mutation assay.

Data Quality: 1, Reliable without restrictions

References: Benzoflex 9-88. Mammalian Cell Mutation Assay. Huntingdon Life Sciences. 1998.

MAMMALIAN CHROMOSOME ABERRATION TEST

Test Substance: See boiling point for purity.

Solvent: DMSO

Study Type: *In-vitro* Mammalian Chromosome Aberration Test in CHL cells

Test Method: OECD 473

GLP: Yes
Date: 1997
Laboratory: Huntingdon Life Sciences
Species/Strain: Chinese Hamster Lung, strain JCRB0030
Concentrations: 4.9, 9.8, 19.5, 39.1, 78.1, 156.3, 312.5 and 625 µg/ml
Metabolic Activation: Sprague-Dawley rat liver
Quantity of Activator: 1.25 and 5 ml
Induction: Stimulated by Aroclor 1254

Criteria for Evaluating Results:

Aberrations were scored according to the classification of ISCN (1985). An International System for Human Cytogenetic Nomenclature, Harden, DG and Klinger, HP (Eds). S. Karger AG, Basel

Positive/Negative Controls: Positive without S9:
Mitomycin C in sterile deionized water
Positive with S9:
Cyclophosphamide in sterile deionized water

Results:

Genotoxic Effects: No statistically significant increases in the proportion of aberrant cells, when compared to the solvent control, were seen in either the presence or the absence of S9 mix. A small response seen in the first test, with S9 mix, was not reproduced in the repeat test or at the later harvest. This response was not considered to be indicative of clastogenic activity.

Data Quality: 1, Reliable without restrictions

References: Benzoflex 9-88. *In-vitro* Mammalian Chromosome Aberration Test in CHL cells. Huntingdon Life Sciences. 1998.

REPEATED DOSE TOXICITY

Test Substance: See boiling point for purity.

Vehicle: Administered by dietary admixture

Study type: Dietary Administration for 13 weeks with a subsequent 4-week recovery period for selected animals

Method: OECD 408
GLP: Yes
Date: 1999
Laboratory: Huntingdon Life Sciences
Species/Strain: Crl: (IGS) CD® BR
Age at Study Initiation: ~ 7-8 weeks
Route of Administration: Dietary
Frequency of Treatment: continuous in diet
Duration of Test: 13 weeks with subsequent 4 week recovery for selected animals
Dose/Concentration Levels: Control, 250, 1000, 1750 OR 2500 mg/kg/day 10 male and 10 female per group
Post-Exposure Observation: An additional 10 male and 10 female rats at the control and 2500 mg/kg/day level served as a recovery group for a subsequent 4 weeks.

Clinical Observations Performed: Individual animals were observed and palpated at least once daily for any signs of behavioral changes, reaction to treatment or ill health. A detailed clinical observation was performed daily for the first 4 weeks of the study. After four weeks of treatment, as treatment related clinical findings were not noted, the frequency of detailed clinical observations was reduced to once per week. The weight of each rat was recorded at the time of allocation of animals to groups, on the day of commencement of treatment and once a week thereafter, including the day of death. The quantity of food consumed by each cage of rats was recorded weekly. Food conversion ratios were calculated, where possible, from the weekly bodyweight and food consumed per unit gain in bodyweight. At weekly intervals, the group mean achieved intake of test substance (mg/kg/day) was calculated from the group mean bodyweight and food consumption and the dietary inclusion levels of the test material. Daily monitoring by visual appraisal of the water bottles was maintained throughout the study. The eyes of all rats were examined using a Keeler indirect opthalmoscope before dosing commenced. During Week 13 the eyes of all animals in the Main Group were examined. As there was no effect of treatment, further investigations were not performed.

RESULTS:

NOAEL: 1000 mg/kg/day or below are considered to represent a No Observable Adverse Effect level of Benzoflex 9-88 in rats by oral administration for 13-weeks.

Actual Dose Received: Achieved Group Mean Intakes of Benzoflex 9-88

Group 2 Males: 245 mg/kg/day
Group 3 Males: 991 mg/kg/day
Group 4 Males: 1714 mg/kg/day
Group 5 Males: 2449 mg/kg/day

Group 2 Females: 246 mg/kg/day
Group 3 Females: 991 mg/kg/day
Group 4 Females: 1713 mg/kg/day
Group 5 Females: 2460 mg/kg/day

Toxic Response/Dose Level:

Mortality: No unscheduled deaths at any dose level
Clinical Signs: No findings which could be conclusively related to treatment. Incidental findings of hairloss and stained fur were noted, however, the incidence showed no correlation to dosage and/or these findings were also noted in the Control group.
Bodyweight: There was a general dosage-related adverse effect on group mean bodyweight gain in both sexes. However, in rats treated with 1000 mg/kg/day or below, the degree of change was insufficient to be of toxicological importance. This is supported by the fact that the final bodyweights for males and females at 1000 mg/kg/day were only 5% less than controls. Small reductions in food intake were insufficient to fully account for the adverse effect on bodyweight. During the 4 week Recovery period, animals previously receiving 2500 mg/kg/day showed vastly superior weight gain than Controls, however 4 weeks appeared to be an insufficient time to completely rectify the effects of 13 weeks of treatment.
Food Consumption: An overall slight, but dosage related, reduction in group mean food consumption was noted for males receiving 1750 or 2500 mg/kg/day. In addition, a slight reduction in group mean food intake was noted for females receiving 2500 mg/kg/day, principally during Week 1 of treatment. The quantities of spilt diet recorded indicated that there may have been a slight adverse palatability reaction in these groups, particularly during Week 1.
Consumption by all other groups, and by rats previously receiving 2500 mg/kg/day during the Recovery period, was considered to be comparable to that of Controls.
Water Consumption: There was no effect of treatment.
Ophthalmoscopy: There were no changes at Week 13 considered to be related to treatment. All findings were characteristic of the age and strain of animals employed. Ophthamoscopy was therefore not performed during the recovery period.
Hematology: Several parameters at Week 13 achieved levels of statistical significance as detailed below, however none could be conclusively related to treatment. A number of erythrocyte parameters achieved levels of statistical significance in Week 13 for rats receiving 1000 mg/kg/day or above (increased red blood cell counts, reduced mean corpuscular volume and reduced mean corpuscular hemoglobin). There were, however, few indications of a dosage-relationship and most individual values were either within the range encountered in Controls or were generally characteristic of rats at this age (within the range of background data at Huntingdon). These minor intergroup differences are not considered to be of toxicological importance. Similarly, levels of statistical significance were achieved for clotting tests (PT, TT and APTT) in one or both sexes at 1000 mg/kg/day or above. The only indication of a dosage-relationship was for a reduction in male TT and APTT data, but with the exception of a low TT for one rat at 1750 mg/kg/day and one rat at 2500 mg/kg/day, all individual values were characteristic of this species. Intergroup clotting test differences are therefore considered to be coincidental. Individual leukocyte data for males receiving 2500 mg/kg/day at Week 13 were generally towards the lower end of the normal range. Although statistical significance was achieved for group mean values, the lowest single result at Week 13 was for a Control animal. In addition, group mean leukocyte data of females receiving 2500 mg/kg/day generally exceeded that of Controls. The slightly lower group mean results for males therefore cannot be conclusively attributed to treatment. There were no other notable findings at Week 13 and all data at Recovery Week 4 was unremarkable.
Biochemistry: Levels of plasma AP activity were raised to statistical significance for male group mean values at 1750 or 2500 mg/kg/day. By recovery Week 4, all AP activities of surviving animals had returned to a level comparable to that of Controls. There were no similar effects of treatment in females. GPT levels were considered to be increased in males receiving 1750 or 2500 mg/kg/day. In addition, there was a dosage-related increase in plasma GOT and OCT activities in males receiving 1000 mg/kg/day or above. In treated female groups, only those receiving 1750 or 2500 mg/kg/day are considered to have shown raised GOT activities; other transaminase levels were unaffected. These findings are considered to be related to treatment, but were only of toxicological importance in rats treated with 1750 or 2500 mg/kg/day, where related hepatic pathology was detected. All animals, except one, previously receiving 2500 mg/kg/day had enzyme activities comparable to concurrent Controls, in Recovery Week 4. This indicates a complete reversibility of treatment-related changes. Group mean plasma cholesterol was increased to a statistically significant degree in males receiving 100 mg/kg/day or above (but without a dosage relationship). A number of individuals in each of these groups showed high levels. This finding was considered to be related to treatment but was only of toxicological importance at 1750 mg/kg/day and above. The group mean cholesterol level of females treated with 2500 mg/kg/day was slightly in excess of Controls and achieved statistical significance; however, in the absence of a dosage-relationship, this was

considered not to be of toxicological importance. In Recovery Week 4, all cholesterol levels were considered normal. No other findings were considered to be of toxicological importance.
Gross pathology: The incidence and distribution of macroscopic findings was not consistent with an effect of treatment.
Organ Weight Changes: There was a slight treatment related increase in liver weight for females receiving 1750 mg/kg/day and males treated with 2500 mg/kg/day. Females receiving 2500 mg/kg/day showed a more marked increase in liver weight. These changes may be partially associated with increased plasma transaminase levels and the low-grade hepatic hypertrophy detected microscopically, however there was little consistency between these data, the degree of hepatic hypertrophy is considered to be low. The group mean liver weight adjusted for bodyweight was increased for male and female rats receiving 2500 mg/kg/day and female rats receiving 1750 mg/kg/day. In the absence of corroborative histopathology or other indicators of target organ toxicity, all statistically significant relative organ weight changes, other than the liver, are considered to be coincidental and of no toxicological importance. The improved bodyweight performance of rats previously receiving 2500 mg/kg/day during the Recovery period lessened the impact of bodyweight upon relative organ weight parameters at the Recovery kill.
Histopathology: Liver: A dose-related incidence and degree of periportal hepatocyte hypertrophy was seen in male and female rats receiving 1750 and 2500 mg/kg/day. No treatment related changes were detected in rats receiving 2500 mg/kg/day and allowed a 4 week recovery period. Periportal hepatocyte hypertrophy is therefore considered to be reversible. Spleen: Hemosiderosis was observed in male and female rats receiving 2500 mg/kg/day and in a small number of female rats receiving 1750 mg/kg/day. The degree of hemosiderosis in male rats receiving 2500 mg/kg/day and allowed a 4 week recovery period was comparable to Controls. A slight degree of hemosiderosis was seen in a smaller number of female rats receiving 2500 mg/kg/day and allowed a 4 week recovery period compared to females of this group at the end of treatment. The absence of any treatment related effect among treated males and the lower incidence of slight hemosiderosis among treated females was considered to show reversibility of this finding. Caecum: An increased incidence of minimal epithelial hyperplasia was seen in male and female rats receiving 2500 mg/kg/day compared to Controls. No treatment related changes were detected in rats receiving 2500 mg/kg/day and allowed a 4-week Recovery period. Epithelial hyperplasia in the caecum is therefore considered reversible.

Statistical Analysis: All statistical analyses were carried out separately for males and females. Data relating to food and water consumption were analyzed on a cage basis. For all other parameters, the analyses were carried out using the individual animal as the basic experimental unit. Food consumption data were analyzed using cumulative totals and water consumption data were analyzed as the total recorded intake over selected time period, expressed on a weekly basis. Bodyweight data were analyzed using weight gains.

Interpretation/Conclusion: Benzoflex 9-88 was administered to rats by dietary admixture to achieve dosages of 0, 250, 1000, 1750 or 2500 mg/kg/day over 13 weeks. Selected Control and Group 5 animals were subsequently maintained off dose for 4 weeks to assess reversibility of any treatment related changes.
Dosages of 1000 mg/kg/day or below are considered to represent a No Observable Adverse Effect Level (NOAEL) of Benzoflex 9-88 in rats by oral administration over 13 weeks. A few minor intergroup differences were noted at 1000 mg/kg/day but were insufficient to be of toxicological importance.
Higher dosages of 1750 or 2500 mg/kg/day were tolerated but the adverse effect on bodyweight was more pronounced, there were increases in circulating enzyme activities, low grade hepatocyte hypertrophy and an increased incidence and degree of hemosiderosis in the spleen in one or both sexes. At 2500 mg/kg/day, an increased incidence of minimal epithelial hyperplasia was noted in the caecum.
When selected animals previously receiving 2500 mg/kg/day were maintained off dose for 4 weeks, all treatment related effects showed evidence of, or complete, recovery.

Data Quality: 1, Reliable without restrictions

References: Benzoflex 9-88. Toxicity to rats by Dietary Administration for 13 weeks with Subsequent 4-Weeek Recovery Period for Selected Animals. Huntingdon Life Sciences. 1999.

Other Studies: IRDC. 1975. No treatment related effects were observed in a 90-day study with dogs administered 1,000 ppm Benzoflex 9-88 in the diet.
Hazleton Laboratories. 1956. Mild reduction in weight gain at 6,000 and 12,000 ppm but no treatment related effects in rats at 5,000 or 10,000 ppm in the diet for 90 days. Mortality was observed at 40,000 ppm.

Developmental Toxicity

Test Substance:		
	Dipropylene Glycol Dibenzoate	94.84%
	Dipropylene Glycol Monobenzoate	2.91%
	Propylene Glycol Dibenzoate	1.7869%
	2(2-Propylenoxy)1-propyl Benzoate	0.2976%

Method: US EPA 870.3700 with the following exception – the guideline states that "Evaluation of the dams during cesarean section and subsequent fetal analyses should be conducted without knowledge of treatment group in order to minimize bias". Evaluation was made with knowledge of treatment group, as procedures are already in place to minimize bias during these portions of the study. These procedures include routine reviews of necropsy technicians evaluation skills and scientific peer review of at least 25% of the raw data of the fetal analyses, including examination of serial sections for visceral anomalies and examination of fetal skeletons.

GLP: Yes

Date: 1998

Laboratory: Huntingdon Life Sciences

Species/Strain: Rat Sprague Dawley

Route of Administration: Oral (gavage)

Dosages: 0, 250, 500 and 1000 mg/kg/day

Number and Sex: 22 Females/group

Exposure period: Gestation Day 6-19

Frequency of Treatment: Daily

Control Group: Corn Oil Vehicle

Duration of Test: Cesarean section on Gestation Day 20

Statistical Evaluation: Statistical tests, employing analysis of variance followed by an inter-group comparison with the Control, were performed on the following parameter: Bodyweight change, bodyweight change adjusted for gravid uterine weight, food consumption, litter data, litter weight, fetal weight and placental weight.

Dependant on the heterogeneity of variance between treatment groups, parametric tests (analysis of variance Snedecor and Cochran 1967) followed by Williams' test (Williams 1971/2) or nonparametric tests (Kruskal-Wallis, Hollander and Wolfe 1973) followed by Shirley's test (Shirley 1977) were used to analyze

these data, as appropriate. Where 75% or more of the values for a given variable, were the same, a Fisher's exact test (Fisher 1950) was used.

For litter data (excluding fetal, litter and placental weights) and implantation loss, due to the preponderance of non-normal distributions, non-parametric tests are generally the most consistent and were routinely used.

All significant (i.e.p<0.5) inter-group differences from the Control are reported only where supported by a significant analysis of variance (i.e. p<0.0.5)

FISHER, R.A. (1950) Fisher's exact test 2x2 contingency table: *Statistical Methods for Research Workers*, para. 21.02 Oliver and Boyd, Edinburgh.

HOLLANDER, M and WOLFE, D.A. (1973) *Non-parametric statistical methods*. Publ. J. Wiley and Sons, New York. KRUSKAL-WALLIS and JONCKHEERE tests: pages 114-132.

SHIRLEY, E. (1977) A non-parametric equivalent of William's test for contrasting increasing dose levels of a treatment. *Biometrics*, 33: 386-389.

SNEDECOR, G.W. and COCHRAN, W.G. (1967) *Statistical methods.* 6th ed. The Iowa State University Press.

WILLIAMS, D.A. (1971/2) William's test for comparing the effect of increasing doses of substance with a zero dose. *Biometrics* 27: 103-117. *Biometrics*, 28: 519-531.

REMARKS

Age at Study Initiation: 10 to 11 weeks of age
Test Substance Preparation: Formulated in corn oil. Formulations prepared fresh each week and refrigerated at approximately 4°C prior to use
Clinical Observation (Maternal): Observed at least twice daily throughout study for any visible signs of reaction to treatment. Observations associated with dosing were also recorded during the treatment period according to the following schedule: 1) predosing, 2) On return of animal to home cage, 3) Afterdosing each group, 4) 1 to 2 hours after completion of dosing all groups, 5) as late as possible in the working day. Maternal bodyweight was measured on Days 0,3, 6 to 17 inclusive and 20 after mating. Food consumption was recorded for the periods Days 0-2, 3-5, 6-8, 9-11, 12-14, 15-16 and 17-19 after mating.
Mating Procedure: Females were paired on a one-to-one basis with stock males of the same strain. Each morning following pairing, the trays beneath the cages were checked for ejected copulation plugs and a vaginal smear was prepared from each female and examined for presence of spermatozoa. The day on which a sperm positive vaginal smear or at least 3 copulation plugs were found was designated Day 0 of gestation.
Terminal Observations (Maternal): On Day 20 after mating, the females were killed by inhaled carbon dioxide for examination of their uterine contents. Each animal was first weighed and then examined macroscopically for evidence of disease or adverse reaction to treatment and specimens of abnormal tissue were retained. The reproductive tract, complete with ovaries, was dissected out and the following recorded: 1) Gravid uterine weight – uterus with cervix, 2) number of corpora lutea in each ovary (assessed prior to removal), 3) number of implantation sites, 4) number of resorption sites (classified as early or late), 5) number and distribution of fetuses in the uterine horn.
Fetal Examination: Each fetus was weighted, sexed and examined for any external abnormalities. Individual placental weights and placental abnormalities were recorded. Fetuses were killed by chilling on a cool plate. The neck and thoracic and abdominal cavities of approximately half of each litter were dissected and examined. Fetal changes were recorded and the offspring eviscerated prior to fixation in Industrial Methylated Spirit. After fixation, fetuses were processed, stained with Alizarin Red and skeletal development assessed. The remaining fetuses in each litter were placed in Bouin's fixative, subjected to free hand serial sectioning and examination for visceral changes.

RESULTS

Maternal Toxicity

NOEL: 1000 mg/kg/day

Clinical Signs: The general condition of females at all dosages remained satisfactory throughout the study and there were no deaths. Salivation after dosing was observed at all dosages. The incidence was dosage related but this finding was not considered to be of toxicological importance. At 1000 mg/kg/day, there were no detectable signs of maternal toxicity; there were no maternal deaths and all females had a live litter at sacrifice.

Litter Responses and Fetal Changes

Prenatal development NOAEL: 500 mg/kg/day. A small number of fetuses with cervical ribs at 1000 mg/kg/day precludes defining this dosage as a NOEL for developmental anomalies, in all other respects the NOAEL for pre-natal development is concluded to be 1000 mg/kg/day.

Fetal Growth and Development NOEL: 250 mg/kg/day

There were no effects of treatment on pre-natal survival or growth.

At 1000 mg/kg/day, treatment was associated with a small but definite increase in the number of fetuses with cervical ribs.

At 1000 and 500 mg/kg/day, there were a greater number of fetuses with incomplete ossification of the 5^{th} and 6^{th} sternebrae compared with Controls, but this finding was not considered to be of any long term toxicological significance.

mg/kg/day	0	250	500	1000
Adult Females				
Females with sperm	22	22	22	22
Pregnant Females	22	22	22	22
Evaluated Pregnant Females	22	22	22	22
Litters – group mean values				
Corpora lutea	16.8	16.3	16.5	16.0
Implantation	15.4	15.4	15.6	15.1
Resorptions	0.9	0.8	0.8	1.0
Live fetuses	14.5	14.6	14.9	14.1
Weight of fetuses (g)				
Male	3.88	3.94	3.87	3.84
Female	3.71	3.75	3.68	3.66
Sex ratios of fetuses (%)				
Male	49.7	51.5	49.6	49.1
Female	50.3	48.5	50.4	50.9
Important Fetal Findings				
Number of fetuses (litters) with:				
Cervical ribs	0 (0)	2 (2)	2 (2)	10 (6)

CONCLUSIONS

At 1000 mg/kg/day, there was no effect of treatment on pre-natal survival or growth. However, skeletal examination revealed a small but definite increase in the incidence of cervical ribs compared with Controls and recent historical background Control data. Therefore, the finding of this skeletal anomaly is considered to be treatment related.

At 500 and 250 mg/kg/day, since the incidence of fetuses with cervical ribs was within the recent background Control data and there was no clear dosage relationship, their occurrence at these dosages is considered likely to be coincidental and unrelated to treatment.

An association between treatment at 1000 and 500 mg/kg/day and the greater number of fetuses with incomplete ossification of the 5th and 6th sternebrae cannot be discounted, particularly since a delay in ossification would be expected to be the most sensitive marker of an effect on pre-natal development where treatment has continued through to the day of sacrifice (treatment period Days 6-19 of gestation). The assessment of fetal ossification on Day 20 of gestation represents a snapshot in time, as the ossification will continue as the animals grow and mature. Although the relationship of these findings to treatment is uncertain, they are considered to be transient in nature, rather than representing permanent structural changes, and therefore are considered to be of no long term toxicological importance.

The increase in cervical ribs at 1000 mg/kg/day is considered to be of greater toxicological significance as it occurred at a dosage which has not produced any detectable signs of maternal toxicity; however, cervical ribs were only found in a small number of fetuses (10/155) at the limit dosage of 1000 mg/kg/day and there were no concomitant changes in vertebral configuration.

In conclusion, it is considered that 1000 mg/kg/day is the no-effect-level for maternal toxicity.

While the occurrence of a small number of fetuses with cervical ribs at 1000 mg/kg/day precludes defining this dosage as a no-observed effect-level for developmental anomalies, in all other respects the no-adverse-effect-level for pre-natal development is concluded to be 1000 mg/kg/day.

The no-adverse-effect-level for all aspects of pre-natal development is concluded to be 500 mg/kg/day.

The no-observed-effect level for fetal growth and development was 250 mg/kg/day.

RELIABILITY: 1, Reliable without restrictions

REFERENCES: Benzoflex 9-88. Study of Prenatal Development in the CD Rat by Oral Gavage Administration. Huntingdon Life Sciences. 2000.

REPRODUCTIVE TOXICITY

Test Substance:	Dipropylene Glycol Dibenzoate	94.84%
	Dipropylene Glycol Monobenzoate	2.91%
	Propylene Glycol Dibenzoate	1.7869%
	2(2-Propylenoxy)1-propyl Benzoate	0.2976%

Method: OECD 416, USEPA OPPTS 870.3800 (1998)

Type: Two generation
GLP: Yes

Date: 1999

Laboratory: Huntingdon Life Sciences

Species/Strain: Rat Sprague Dawley (CD-IGS)

Route of Administration: Dietary – Continuous

Doses: 1000, 3300 or 10000 ppm throughout two generations

Sex: males and females

Control Group: Yes, basal diet without test material

Frequency: continuously in diet

Duration: Approximately 38 weeks

Premating Exposure (males and females F0 and F1): 10 weeks before pairing and throughout mating, gestation, littering and lactation

Statistical Methods:
Where considered appropriate, significance tests employing analysis of variance followed by an intergroup comparison with the control were performed. These were performed on the following parameters: bodyweights and bodyweight change, food consumption, litter data, sexual development data, seminology data, organ weights and histopathological findings.

For data recorded and/or processed by the Xybion computer system (adult organ weights and weekly bodyweight change) for the parental animals, homogeneity of variance was assessed using Barlett's test. Whenever this was found to be statistically significant a Beherens-Fisher test was used to perform pairwise comparison, otherwise a Dunnett's test was used. Intergroup differences in macroscopic pathology and histopathology were assessed using Fisher's test.

For bodyweight and food consumption data during gestation and lactation, litter data, sexual development data, seminology data and offspring organ weights the statistical analysis was performed using the Startox program developed by Huntingdon life Sciences. Dependant on the heterogeneity of variance between treatment groups, parametric tests (analysis of variance, Snedecor and Cochran 1967) followed by Williams' test (Williams' 1971/2) or non-parametric tests (Kruskal- Wallis, Hollander and Wolfe 1973) followed by Shirley's test (Shirley 1977) were used, as appropriate.

Where 75% or more of the values for a given variable were the same, a Fisher's exact test (Fisher 1950) was used.

Significant (i.e. p<0.05) inter-group differences from the Control are reported where supported by a significant analysis of variance (i.e. p<0.05).

FISHER, R.A. (1950) Fisher's exact test 2x2 contingency table: *Statistical Methods for Research Workers*, para. 21.02 Oliver and Boyd, Edinburgh.

HOLLANDER, M and WOLFE, D.A. (1973) *Non-parametric statistical methods*. Publ. J. Wiley and Sons, New York. KRUSKAL-WALLIS and JONCKHEERE tests: pages 114-132.

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SNEDECOR, G.W. and COCHRAN, W.G. (1967) *Statistical methods.* 6th ed. The Iowa State University Press.

WILLIAMS, D.A. (1971/2) William's test for comparing the effect of increasing doses of substance with a zero dose. *Biometrics* 27: 103-117. *Biometrics*, 28: 519-531.

REMARKS

Groups of rats were administered continuously in their diet 1000, 3300 or 10000 ppm of test compound throughout two generations. A fourth group received the basal diet without the test material and served as the Control.

The F0 generation, which comprised 32 males and 32 females in each group, received the treated diet for 10 weeks before pairing and throughout mating, gestation, littering and lactation. Offspring survival, growth and sexual maturation were evaluated. From the litters 28 male and 28 female offspring per group were selected to form the F1 generation. Both sexes received similarly treated diets as their parents for a minimum of 10 weeks from selection, throughout pairing, gestation, littering and lactation. F2 offspring were monitored for survival and development until weaning.

All F0 and F1 adult animals were subjected to detailed necropsy, the reproductive organs and selected organs were weighed and retained. Sperm motility and morphology was assessed from samples obtained from the left vas deferens and sperm counts were determined for the left epididymis and testis for all F0 and F1 males. Histopathological examinations were performed on designated tissues from 10 parent males and 10 parent females in the Control and high dose groups, and abnormal tissues from all other parental animals.

Unselected F1 offspring were killed at 34 Days of age and F2 offspring were killed on Day 21 of age. Where possible, one male and one female from each litter were subjected to necropsy examination, the reproductive organs retained, and the brain, spleen and thymus weighed and retained.

Mating Procedure – After 10 weeks of treatment for the F0 generation and 10 weeks after selection for the F1 generation, males and females from within the same treatment groups were paired on a one-to-one basis for a period up to 3 weeks. If there were no positive indication of mating after 14 days and the females had shown no signs of estrous at the time, the male partner was replaced by a proven male from within the same group. Care was taken to avoid pairing siblings. Each morning following pairing, the trays beneath the cages were checked for ejected copulation plugs and a vaginal smear was prepared from each female and examined for the presence of spermatozoa and the stage of the estrous cycle. The day on which evidence of mating was found was designated Day 0 of gestation.

Once mating occurred, the males and females were generally separated and smearing was discontinued. However after inconclusive mating, smearing continued up to 5 days to confirm positive mating.

Parameters assessed during F0 and F1 – All animals were observed at least twice daily throughout the study and any visible signs of reaction to treatment were recorded. A more detailed weekly examination was performed throughout the treatment period. All animals found dead or killed for reasons of animal

welfare were subjected to a thorough macroscopic examination of the visceral organs and specimens of abnormal tissues were retained. Males were weighed on the day that treatment commenced (F0) or the formal start of the generation (F1), then weekly thereafter. F0 and F1 females were weighed on the same schedule until mating was detected and then on Days 0, 6, 13 and 20 after mating and on Days 1, 4, 7, 14 and 21 of lactation. Food consumption was recorded on a cage basis for F0 and F1 males and females weekly before pairing. Food consumption for females after mating was recorded daily on an individual basis on Days 0-5, 6-12 and 13-19 after mating and on Days 1-3, 4-6, 7-13 and 14-20 of lactation. After Day 14 of lactation, food intake is increasingly influences by the offspring and is no longer an accurate reflection of maternal intake.

Estrous cycles F0 and F1– For 22 days before pairing of F0 and F1 generations, daily vaginal smears were taken from all females and examined to establish the duration and regularity of the estrous cycle. After pairing with the male, smearing was continued until evidence of mating was observed. Following weaning, daily vaginal smears were taken from all females on Days 22 to 28 after birth prior to necropsy and use to determine the stage of the estrous cycle at termination. Females whose litters died before weaning were retained and vaginal smears taken on their theoretical Days 22-28 and killed on Day 28, as for those with surviving litters. Any F0 females that failed to mate, mated but were not pregnant or failed to litter were retained and vaginal smears taken for 7 days, starting on the day on which the first batch of females with live litters had vaginal smears taken. These animals were killed with the first batch of females with a litter. Any F1 females that failed to mate, mated but were not pregnant or failed to litter were retained and vaginal smears taken for 9 days, starting on the day on which the first batch of females with live litters had vaginal smears taken. These animals were killed with the third batch of females with a litter.

Seminology F0 and F1 – After sacrifice, sperm motility, sperm morphology, sperm count and homogenization-resistant spermatids were counted.

Parameters Assessed during F1 and F2 – All offspring were examined at approximately 24 hours after birth (Day 1 of age) and the following were recorded for each litter: number of offspring (live and dead), individual bodyweights of live offspring, sex ratio and observations on individual offspring. Litters were observed daily for evidence of abnormal appearance or behavior. Daily records were maintained of mortality and consequent changes in litter size. Whenever possible, any offspring found dead were examined externally and internally. Litters containing more than 10 offspring were culled by random selection to 10 (where possible 5 males and 5 females) on Day 4 of age. Individual F1 and F2 offspring were weighed on Days 1,4,7,14 and 21 of age. Selected female F1 generation offspring were examined daily from Day 28 of age until vaginal opening occurred. Bodyweights were recorded on day of vaginal opening for each animal. Selected male F1 generations offspring were examined daily form Day 35 of age until balano-preputial separation occurred. Bodyweight was recorded on day of start and completion of separation for each animal.

All parental animals were subject to a detailed macroscopic examination for evidence of disease or adverse reaction to treatment. The necropsy procedure included a review of the history of each animal, and a detailed examination of the cranial, thoracic, abdominal and pelvic cavities and their viscera. The external and cut surfaces of the organs and tissues were examined, either before or after weighing as appropriate. The number of uterine implantation sites was recorded for the adult females. Abnormalities interactions and changes were noted, the requisite organs weighed and the required tissue samples preserved in fixative. Unselected F1 offspring and F2 offspring were examined macroscopically for evidence of disease or adverse reaction to treatment and appropriate organs weighed and retained. Any abnormal tissues were also retained.

The following tissues were microscopically examined for 10 parent males and 10 parent females of groups 1-4 sacrificed on completion of the schedule treatment period and for all adult animals killed or dying before schedules termination: adrenal glands, epididymis, mammary glands (causal), ovaries with oviduct, pituitary, prostrate (ventral lobe), seminal vesicles and coagulated gland, testis (right), uterus with cervix, vagina. Mammary glands were retained from females with total litter loss.

RESULTS

The general condition of the F0 to F1 and F1 to F2 generations were satisfactory throughout. There were no treatment related deaths in either generation of adult animals. Bodyweight changes of F0 females before pairing and F1 males were slightly, but significantly, lower than in Controls. No adverse effects were seen in overall parental food consumption; food conversion efficiency calculated during the 10 week premating phase was considered similar to controls for both generations. The achieved dosage at all dietary concentrations for both sexes was considered satisfactory and exposure to the test material at over 500 mg/kg/day was achieved in the top dose group prior to pairing. Exposure to the test material during the key phases of gestation and peak lactation was around 800 and 1500 mg/kg/day respectively.

Estrous cycles, mating performances, fertility and fecundity were similar in all groups. Gestation length and the parturition process were unaffected by treatment. Assessment of terminal vaginal smears taken from the F0 females revealed a higher incidence of females in estrous in groups treated with the test material compared with controls. This finding was not apparent among F1 females and is considered to be of doubtful biological significance.

Litter parameters at birth of the F1 and F2 progeny and their survival to weaning showed no apparent detrimental effects to treatment. However, in both the F1 and F2 offspring at 10,000 ppm there was slight reduction in weight gain during Days 14-21 of age and this finding may be linked to the transition to direct exposure to the test material as the offspring weaned onto solid diet at the same dietary inclusion levels as their parents.

No treatment related findings were seen at macroscopic examination of the F1 offspring not selected to form the next generation or the F2 offspring killed after weaning.

Macropathology, histopathological assessment and sperm analysis for the F0 and F1 adults showed no adverse effects of treatment.

The only possible effect of treatment detected at assessment of organ weights from F1 and F2 offspring was significantly lower absolute and bodyweight relative spleen weights among F2 males and females compared with Controls. The toxicological significance of this finding is uncertain since it was not detected among F1 offspring or among F0/F1 adult animals.

The NOEL is 10,000 ppm for F0 and F1 parent animals and the NOAEL for survival and growth of offspring is considered to be 10,000 ppm.

Dietary Concentration ppm	0	1000	3300	10000
F0 Parental Animals				
Number females with normal estrous cycle	30	31	30	30
Number males/females paired 1:1	32	32	32	32
Number females with sperm	32	32	32	32
Pregnant females	31	30	31	30
Females with delivery	31	29	31	30
Important parental findings				
None to be considered related to treatment				
F1 Parental Animals				
Number females with normal estrous cycles	26	27	24	26
Number male/female paired 1:1	28	28	28	28
Number females with sperm	27	28	27	28
Pregnant females	26	26	26	28
Females with delivery	26	26	26	28
Important parental findings				
None considered to be related to treatment				
F1 Litters				
Implantations, assessed at termination mean	15.2	15.1	15.1	15.1
Live litters	31	29	31	30
Sex ratio day 1 after birth (as %M) mean	53.7	49.2	49.2	52.4
Number offspring Day 4 after birth (before culling) mean	10.7	12.4	12.5	12.8
Number offspring Day 4 after birth (after culling) mean	8.7	9.7	9.7	9.5
Surviving litters at Day 4 after birth	29	28	27	29
Number offspring at day 21 mean	8.8	9.5	9.6	9.6
Surviving litters at day 21 after birth	25	28	27	26
Weight at birth (g) mean				
Males	6.0	6.1	6.1	6.1
Females	5.6	5.7	5.7	5.7
Weight at weaning (g) mean				
Males	48.5	47.0	47.7	44.0
Females	46.1	44.3	44.9	41.9
Important findings for F1 post-weaning progeny – to sexual maturation				
None considered to be related to treatment				
F2 Litters				
Implantations, assessed at termination mean	15.4	14.7	13.7	13.9
Live litters	26	26	26	28
Sex ratio Day 1 after birth (as % M) mean	50.5	52.7	44.1	50.6
Surviving litters at day 4 after birth	24	22	25	25
Number offspring Day 4 after birth (before culling) mean	13.3	12.0	12.2	12.9
Number offspring Day 4 after birth (after culling) mean	9.7	9.4	9.4	9.6
Surviving litters at Day 21 after birth	24	22	25	25
Number offspring at Day 21 mean	9.1	8.5	9.3	9.5
Weight at birth (g) mean				
Males	6.2	6.1	6.4	6.3
Females	5.8	5.7	6.1	5.9

Weight at weaning (g) mean				
Males	48.6	43.2	47.4	45.1
Females	46.6	43.3	45.6	43.5
Important findings for F2 progeny				
Bodyweight relative spleen weights (% bodyweight) on Day 21 of age				
Males	0.4609	0.4102	0.4357	0.3736
Females	0.4802	0.4436	0.4654	0.4081

Conclusion

Dietary administration of the test material at concentrations of 1000, 3300 or 10000 ppm was well tolerated by the F0 and subsequent F1 parental animals and their respective progeny. Predicted exposure to the test material was achieved throughout both generations of the study as judged by calculations of achieved intake and period dietary analysis. There were no obvious toxicological effects of treatment for the two generations on the general condition of the parental animals or on their fertility and reproductive performance.

Litter parameters at birth of the F1 and F2 progeny and their survival to weaning showed no apparent detrimental effects of treatment. However, in both F1 and F2 offspring at 10,000 ppm there was a slight reduction in weight gain during Days 14-21 of age and this finding may be linked to the transition to direct exposure to the test material as the offspring weaned onto solid diet at the same dietary inclusion levels as their parents.

No abnormal findings were apparent at necropsy of the F0 or F1 parental animals, the post-weaned unselected F1 offspring or the F2 offspring. Organ weight assessment of the F0 and F1 parent animals did not suggest any adverse effects on any organs. Assessment of spermatogenesis and histopathology in both parental generations showed that there were no injurious effects on these testes or other reproductive organs. Furthermore, detailed histopathological examination of these tissues from both sexes in both generations did not reveal any adverse effects of treatment. Regarding survival and growth of the offspring, there were no unequivocal adverse effects. However, a slight reduction in bodyweight gain during Days 14 to 21 (F1 and F2), likely due to the neonatal consumption of the dam's treated diet, and a slight reduction in spleen weights only observed in the F2 generation are of questionable toxicological relevance.

The evidence from this study suggested that a dietary concentration of 10,000 ppm should be considered as the No-Observed-Effect-Level (NOEL) for the F0 and F1 parent animals. The No-Observed-Adverse-Effect-Level (NOAEL) for survival and growth of the offspring is considered to be 10,000 ppm.

RELIABILITY: 1, Reliable without restrictions

REFERENCES: Benzoflex 9-88. Study of Reproductive Performance in CD Rats Treated Continuously through Two Successive Generations by Dietary Administration. Huntingdon Life Sciences. 2001.

ADDITIONAL STUDIES

International Research and Development Corporation.

Five male and five female rats were placed in a sealed 59.1 liter glass chamber and exposed for 4 hours to a dynamic atmosphere containing Benzoflex 9-88 mist/vapor. The calculated atmospheric concentration administered was approximately 200 mg/L (mist/vapor) of Benzoflex 9-88. All the rats exposed to the 200-mg/L (mist/vapor) atmospheric concentration of Benzoflex 9-88 survived the 4-hour exposure period and the 14-day observation period. Benzoflex 9-88 would not be considered harmful if inhaled.

Benzoflex 9-88. Eye Irritation to the Rabbit. Huntingdon Life Sciences. 1998.

OECD 404. None of the treated animals showed a positive response. No corneal damage or iridial inflammation was observed. Transient hyperemia of blood vessels only was observed in all animals. These reactions had resolved in all instances by one or two days after instillation.

Benzoflex 9-88. Skin Irritation to the Rabbit. Huntingdon Life Sciences. 1998.

OECD 404. A single semi-occlusive application of Benzoflex 9-88 to intact rabbit skin for four hours elicited no dermal irritation.

Benzoflex 9-88. Skin Sensitization to the Guinea Pig. Huntingdon Life Sciences. 1998.

OECD 406. Benzoflex 9-88 did not produce evidence of skin sensitization (delayed contact hypersensitivity) in any of twenty test animals. Evidence of skin sensitization was produced by hexyl cinnamic aldehyde (HCA) in all ten positive controls thus confirming the sensitivity of the method.

Benzoflex 9-88. Acute Toxicity (LC_{50}) to the Earthworm (*Eisenia foetida)*. Huntingdon Life Sciences. 1998.

OECD 207. Under the conditions of this study, the LC_{50} of Benzoflex 9-88 to the earthworm was found to be in excess of 1000 ppm. The NOEL was considered to be 1000 ppm.

Evaluation of Velsicol Benzoflex 2-45 and Benzoflex 9-88 Plasticizers for Estrogenic Activity Using Vaginal Cornification and the Uterotrophic Response in the Ovariectomized Adult Rat as the Endpoints. BIOQUAL, Inc. 1997. Benzoflex 9-88 did not induce vaginal cornification at doses of 500, 1000, 1500 or 2000 mg/kg/day for 7 days by oral gavage in ovariectomized adult Spraque-Dawley (CD) rats. Benzoflex 9-88 did not stimulate a uterine weight increase or an increase in the uterine weight to final body weight ratio at doses of 500, 1000, 1500 or 2000 mg/kg/day for 7 days. When compared with the vehicle control (corn oil) and positive control (diethylstilbestrol), these data demonstrate that Benzoflex 9-88 did not exhibit estrogenic activity up to and including the maximally tolerated dose.

Butz RG, Atallah YH, Yu CC and Calo CJ. Environmental Toxicology and Chemistry. 1, 337. 1982. Studies conducted show Benzoflex 9-88 is rapidly metabolized and excreted from the body. It does not accumulate in rats and this behavior is expected in other mammalian systems as well. This conclusion is supported through the test where oral doses of ^{14}C-labeled Benzoflex 9-88 were rapidly absorbed through the gut in rats. Seventy percent of the administered dose was excreted through the urine within 48 hours as hippuric acid, and about 10% was observed in the feces. The half-life of radiocarbon in the blood was 3 hours and for other organs 2-15 hours.